

PATENT APPLICATION  
Mo6314  
LeA 34,326

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF )  
MARCO BUSCH ET AL )  
SERIAL NUMBER: TO BE ASSIGNED )  
FILED: HEREWITH )  
TITLE: METHOD OF FINDING MODULATORS )  
OF ENZYMES OF THE CAROTENOID )  
BIOSYNTHETIC PATHWAY )

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to calculation of the filing fee and examination of the present application,  
please amend the application as follows:

"Express Mail" mailing label number ET146895600US  
Date of Deposit May 2, 2001

I hereby certify that this paper or fee is being deposited with the United States  
Postal Service "Express Mail Post Office to Addressee" service under 37 CFR  
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of Patents and Trademarks, Washington, D.C. 20231

Donna J. Veatch  
(Name of person mailing paper or fee)

Donna J. Veatch  
Signature of person mailing paper or fee)

**In the Specification:**

Please amend the specification as follows:

On page 1, after the title, please insert:

--FIELD OF THE INVENTION--

On page 1, after line 7, please insert:

--BACKGROUND OF THE INVENTION--

On page 4, after line 11, please insert:

--SUMMARY OF THE INVENTION

A nucleic acid which encodes a polypeptide with the bioactivity of a zeta-carotene desaturase, comprises the amino acid sequence of SEQ ID NO: 6.

DETAILED DESCRIPTION--

**In the Claims:**

Please cancel Claims 22-24.

Please amend Claims 1-21 and 25-27 as follows:

1. (Amended) A nucleic acid which encodes a polypeptide from tobacco with the bioactivity of a zeta-carotene desaturase, comprising the amino acid sequence of SEQ ID NO: 6.
2. (Amended) A nucleic acid according to Claim 1, wherein the nucleic acid encodes a polypeptide with the amino acid sequence of SEQ ID NO: 6.
3. (Amended) A nucleic acid according to Claim 1, wherein the nucleic acid is a single-stranded or double-stranded DNA or RNA.
4. (Amended) A nucleic acid according to Claim 3, wherein the nucleic acid is a fragment of genomic DNA or cDNA.

5. (Amended) A nucleic acid according to Claim 1, wherein the nucleic acid is derived from tobacco plants.

6. (Amended) A nucleic acid according to Claim 1, comprising a sequence selected from

- (a) the sequence of SEQ ID NO: 5,
- (b) sequences encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 6,
- (c) partial sequences of the sequences defined under (a) or (b) which are at least 14 base pairs in length,
- (d) sequences which hybridize with the sequences defined under (a), (b) or (c),
- (e) sequences which are complementary to the sequences defined under (a), (b) or (c), and
- (f) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the sequences defined under (a) to (c).

7. (Amended) A regulatory region which naturally controls, in plant cells, the transcription of a nucleic acid according to Claim 1.

8. (Amended) A DNA construct comprising a nucleic acid according to Claim 1 and a heterologous promoter.

9. (Amended) A vector comprising a nucleic acid according to Claim 1.

Table 1. Demographic characteristics of the study population	
Age (years)	Mean (SD)
Male	55.2 (10.5)
Female	56.8 (11.2)
Marital status	
Married	78.5%
Single	12.3%
Divorced	8.2%
Widowed	1.0%
Education level	
High school or above	65.4%
Below high school	34.6%
Occupation	
White collar	45.2%
Blue collar	54.8%
Income (USD/month)	
< 1000	15.3%
1000-2000	32.1%
2000-3000	28.7%
> 3000	23.9%
Health insurance	
Yes	89.5%
No	10.5%
Smoking status	
Current smoker	22.1%
Former smoker	18.5%
Non-smoker	59.4%
Alcohol consumption	
Regular drinker	12.8%
Occasional drinker	25.3%
Non-drinker	61.9%
Family size	
1-2	35.2%
3-4	42.1%
5 or more	22.7%
Chronic diseases	
Hypertension	38.5%
Diabetes	15.2%
Heart disease	12.8%
Stroke	8.1%
Arthritis	22.3%
Chronic kidney disease	5.4%
Chronic liver disease	3.2%
Chronic respiratory disease	7.9%
Chronic pain	18.7%
Depression	11.5%
Other chronic conditions	14.3%

12. (Amended) A host cell according to Claim 11, wherein the host cell is a prokaryotic cell.

14. (Amended) A polypeptide with the bioactivity of a phytoene synthase which is encoded by a nucleic acid of SEQ ID NO: 1 or SEQ ID NO: 3, comprising an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.

16. (Amended) An antibody which binds specifically to a polypeptide of Claim 14.

18. (Amended) A process for generating a nucleic acid according to Claim 1, comprising the steps of

(b) chemically synthesizing oligonucleotides, labelling the oligonucleotides, hybridizing the oligonucleotides with DNA of a genomic or cDNA library which

has been generated starting from genomic DNA or mRNA of plant cells, selecting positive clones and isolating the hybridizing DNA from positive clones, or

- (c) chemically synthesizing oligonucleotides and amplifying target DNA by means of PCR.

19. (Amended) A process for generating a polypeptide according to Claim 14, comprising

- (a1) culturing a host cell comprising a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 under conditions which ensure the expression of the nucleic acid, or
- (a2) expressing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 in an *in-vitro* system, and
- (b) obtaining the polypeptide from the cell, the culture medium or the *in-vitro* system.

20. (Amended) A method of finding a chemical compound which binds to a polypeptide according to Claim 14 or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps

- (a) contacting a host cell containing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6, a polypeptide according to Claim 14 or a polypeptide with the bioactivity of a phytoene desaturase with a chemical compound or a mixture of chemical compounds under conditions which permit the interaction of a chemical compound with the polypeptide, and

[illegible]

(a) contacting a host cell containing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 with a chemical compound or a mixture of chemical compounds,

(c) determining the compound which specifically influences the expression of the polypeptide.

26. (Amended) An organism selected from plants, parts of plants, protoplasts, plant tissues or plant propagation materials, wherein the organism comprises a polypeptide according to Claim 14 whose bioactivity or expression pattern is modified in comparison with the corresponding endogenous polypeptides.

27. (Amended) A method of generating plants, parts of plants, protoplasts, plant tissues or plant propagation materials according to Claim 25, comprising the step of modifying a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 by endogenous mutagenesis.

Please add the following claims:

- 28. A vector comprising a regulatory region according to Claim 7.
29. A vector comprising a DNA construct according to Claim 8.
30. A host cell containing a DNA construct according to Claim 8.
31. A host cell containing a vector according to Claim 9.
32. A process for generating a polypeptide according to Claim 15, comprising
- (a1) culturing a host cell comprising a nucleic acid comprising a sequence selected from
- (i) the sequence of SEQ ID NO: 5,
  - (ii) sequences encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 6,
  - (iii) sequences which hybridize with the sequences defined under (i) or (ii),
  - (iv) sequences which are complementary to the sequences defined under (i) or (ii), and
  - (v) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the sequences defined under (i) or (ii);
- in a culture medium under conditions which ensure the expression of the nucleic acid, or
- (a2) expressing a nucleic acid which encodes a polypeptide with the bioactivity of a zeta-carotene desaturase, comprising the amino acid sequence of SEQ ID NO: 6 in an *in-vitro* system, and

- (b) obtaining the polypeptide from the cell, the culture medium or the *in-vitro* system.

33. A method of finding a chemical compound which binds to a polypeptide according to Claim 15 or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps:

- (a) contacting a host cell comprising a nucleic acid which encodes a polypeptide with the bioactivity of a zeta-carotene desaturase, comprising the amino acid sequence of SEQ ID NO: 6, a polypeptide according to Claim 15 or a polypeptide with the bioactivity of a phytoene desaturase with a chemical compound or a mixture of chemical compounds under conditions which permit the interaction of a chemical compound with the polypeptide, and
- (b) determining the chemical compound which binds specifically to the polypeptide.

34. A method of finding a compound which modifies the expression of polypeptides according to Claim 15 or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps:

- (a) contacting a host cell containing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: with a chemical compound or a mixture of chemical compounds,
- (b) determining the polypeptide concentration, and
- (c) determining the compound which specifically influences the expression of the polypeptide.

35. An organism selected from transgenic plants, parts of plants, protoplasts, plant tissues and plant propagation materials, wherein the organism



comprises an intracellular concentration of a polypeptide according to Claim 17 which is increased or reduced in comparison with the corresponding wild-type cells after introducing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

36. An organism selected from plants, parts of plants, protoplasts, plant tissues or plant propagation materials, wherein the organism comprises a polypeptide according to Claim 15 whose bioactivity or expression pattern is modified in comparison with the corresponding endogenous polypeptides.

37. A method of generating plants, parts of plants, protoplasts, plant tissues or plant propagation materials according to Claim 25, comprising the step of modifying by endogenous mutagenesis a regulatory region which naturally controls, in plant cells, the transcription of a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

38. A nucleic acid comprising a sequence selected from the group consisting of:

- (a) the sequences of SEQ ID NOS: 1, 3 and 5,
- (b) sequences encoding polypeptides which comprises the amino acid sequences of SEQ ID NOS: 2, 4, and 6, and
- (c) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the amino acid sequences of SEQ ID NOS: 2, 4, and 6.--

## **REMARKS**

By present amendment the claims have been amended to present the claims in accordance with customary U.S. practice, care having been exercised to avoid any introduction of new matter.

Claims 22-24 have been canceled, and Claims 1-21 and 25-27 have been amended as to form. Claims 28-38 have been added. Support for Claims 28 and 29 can be found in Claim 9, while support for Claims 30 and 31 can be found in Claim 11. Support for Claims 32-37 can be found in Claims 19, 20, 21, 25, 26, and 27, respectively. Support for Claim 38 can be found in the specification on page 6, lines 8-25. The amendments to the claims remove multiple dependencies and place the claims in United States form.

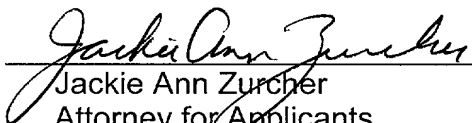
The specification has been amended to include section headings in accordance with customary U.S. practice, and to include a Summary of the Invention. Support for the amendment to the specification can be found in original Claim 1.

The amendments to the claims and the specification do not involve any introduction of new matter, whereby entry is believed to be in order and is respectively requested.

Attached is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version With Markings To Show Changes Made".

A disk containing a computer readable form of the Sequence Listing is enclosed herewith. The information recorded in computer readable form is identical to the Sequence Listing set forth on the paper copy. The attached diskette is submitted in American Standard Code for Information Interchange (ASCII) text.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

**In the Claims:**

Claims 22-24 have been canceled.

Claims 1-21 and 25-27 have been amended as follows:

1. (Amended) A n[N]ucleic acid[s] which encodes a polypeptide[s] from tobacco with the bioactivity of a zeta-carotene desaturase, comprising [which comprises] the amino acid sequence of SEQ ID NO: 6.
2. (Amended) A n[N]ucleic acid[s] according to Claim 1, wherein the nucleic acid encodes a polypeptide [characterized in that they encode polypeptides] with the amino acid sequence of SEQ ID NO: 6.
3. (Amended) A n[N]ucleic acid[s] according to Claim 1, wherein the nucleic acid is a [1 or 2, characterized in that they are] single-stranded or double-stranded DNA or RNA.
4. (Amended) A n[N]ucleic acid[s] according to Claim 3, wherein the nucleic acid is a fragment [characterized in that they are fragments] of genomic DNA or cDNA.
5. (Amended) A n[N]ucleic acid[s] according to Claim 1, wherein the nucleic acid is [one of Claims 1 to 4, characterized in that they are] derived from tobacco plants.
6. (Amended) A n[N]ucleic acid[s] according to Claim 1 [one of Claims 1 to 5], comprising a sequence selected from [amongst]
  - (a) the sequence of SEQ ID NO: 5,

- (b) sequences encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 6,
- (c) partial sequences of the sequences defined under (a) or (b) which are at least 14 base pairs in length,
- (d) sequences which hybridize with the sequences defined under (a), (b) or (c),
- (e) sequences which are complementary to the sequences defined under (a), (b) or (c), and
- (f) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the sequences defined under (a) to (c).

7. (Amended) A<sub>r</sub>[R]egulatory region which naturally controls, in plant cells, [in particular in tobacco plants, ]the transcription of a nucleic acid according to Claim 1 [one of Claims 1 to 6].

8. (Amended) A DNA construct comprising a nucleic acid according to Claim 1 [one of Claims 1 to 6 ]and a heterologous promoter.

9. (Amended) A<sub>v</sub>[V]ector comprising a nucleic acid according to Claim 1 [one of Claims 1 to 6, a regulatory region according to Claim 7 or a DNA construct according to Claim 8].

10. (Amended) A<sub>v</sub>[V]ector according to Claim 9, wherein [characterized in that] the nucleic acid is linked functionally to regulatory sequences which ensure the expression of the nucleic acid in pro- or eukaryotic cells.

11. (Amended) A<sub>h</sub>[H]ost cell containing a nucleic acid according to Claim 1 [one of Claims 1 to 6, a DNA construct according to Claim 8 or a vector according to Claim 9 or 10].

12. (Amended) A h[H]ost cell according to claim 11, wherein the host cell [characterized in that it] is a prokaryotic cell[, in particular *E. Coli*].

13. (Amended) A h[H]ost cell according to Claim 11, wherein the host cell [characterized in that it] is a eukaryotic cell[, in particular a yeast cell, insect cell, mammalian cell or plant cell].

14. (Amended) A p[P]olypeptide with the bioactivity of a phytoene synthase which is encoded by a nucleic acid of SEQ ID NO: 1 or SEQ ID NO: 3, comprising an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4.

15. (Amended) A p[P]olypeptide with the bioactivity of a zeta-carotene desaturase which is encoded by a nucleic acid according to Claim 1 [one of Claims 1 to 6].

16. (Amended) An a[A]ntibody which binds specifically to a polypeptide of Claim 14.

17. (Amended) An a[A]ntibody which binds specifically to a polypeptide of Claim 15.

18. (Amended) A p[P]rocess for generating a nucleic acid according to Claim 1, comprising the steps of [one of Claims 1 to 6, comprising the following steps]:

- (a) completely chemically synthesizing the nucleic acid [synthesis carried out in a manner known per se] or
- (b) chemically synthesizing [synthesis of] oligonucleotides, labelling the oligonucleotides, hybridizing the oligonucleotides with DNA of a genomic or cDNA library which has been generated starting from genomic DNA or mRNA

of plant cells, selecting [selection of] positive clones and isolating [isolation of] the hybridizing DNA from positive clones, or

- (c) chemically synthesizing [synthesis of] oligonucleotides and amplifying [amplification of the] target DNA by means of PCR.

19. (Amended) A p[P]rocess for generating a polypeptide according to Claim 14 [or 15], comprising

- (a1) culturing a host cell comprising a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 [according to one of Claims 11 to 13] under conditions which ensure the expression of the nucleic acid [according to one of Claims 1 to 6], or
- (a2[b]) expressing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 [according to one of Claims 1 to 6] in an *in-vitro* system, and
- (b[c]) obtaining the polypeptide from the cell, the culture medium or the *in-vitro* system.

20. (Amended) A m[M]ethod of finding a chemical compound which binds to a polypeptide according to Claim 14 [and/or 15] or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps

- (a) contacting a host cell containing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 [according to one of Claims 11 to 13], a polypeptide according to Claim 14 [or 15] or a polypeptide with the bioactivity of a phytoene desaturase with a chemical compound or a mixture of chemical compounds under conditions which permit the interaction of a chemical compound with the polypeptide, and

- (b) determining the chemical compound which binds specifically to the polypeptide.

21. (Amended) A m[M]ethod of finding a compound which modifies the expression of polypeptides according to Claim 14 [or 15] or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps:

- (a) contacting a host cell containing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 [according to one of Claims 11 to 13] with a chemical compound or a mixture of chemical compounds,
- (b) determining the polypeptide concentration, and
- (c) determining the compound which specifically influences the expression of the polypeptide.

25. (Amended) An organism selected from t[T]ransgenic plants, parts of plants, protoplasts, plant tissues and [or] plant propagation materials, wherein the organism comprises an [characterized in that the] intracellular concentration of a polypeptide according to Claim 16 [or 17] which is increased or reduced in comparison with the corresponding wild-type cells after introducing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 [according to one of Claims 1 to 6, a DNA construct according to Claim 8 or a vector according to Claim 9].

26. (Amended) An organism selected from p[P]lants, parts of plants, protoplasts, plant tissues or plant propagation materials, wherein the organism comprises [characterized in that they contain] a polypeptide according to Claim 14 [or 15] whose bioactivity or expression pattern is modified in comparison with the corresponding endogenous polypeptides.



27. (Amended) A method of generating plants, parts of plants, protoplasts, plant tissues or plant propagation materials according to Claim 25, comprising the step of modifying a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6 by endogenous mutagenesis [characterized in that a nucleic acid according to one of Claims 1 to 6 or a regulatory region according to Claim 7 is modified by endogenous mutagenesis].

Claims 28-38 are new and have been added as follows:

--28. A vector comprising a regulatory region according to Claim 7.

29. A vector comprising a DNA construct according to Claim 8.

30. A host cell containing a DNA construct according to Claim 8.

31. A host cell containing a vector according to Claim 9.

32. A process for generating a polypeptide according to Claim 15, comprising

(a1) culturing a host cell comprising a nucleic acid comprising a sequence selected from:

- (i) the sequence of SEQ ID NO: 5,
- (ii) sequences encoding a polypeptide which comprises the amino acid sequence of SEQ ID NO: 6,
- (iii) sequences which hybridize with the sequences defined under (i) or (ii),
- (iv) sequences which are complementary to the sequences defined under (i) or (ii), and
- (v) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the sequences defined under (i) or (ii);

in a culture medium under conditions which ensure the expression of the nucleic acid, or

- (a2) expressing a nucleic acid which encodes a polypeptide with the bioactivity of a zeta-carotene desaturase, comprising the amino acid sequence of SEQ ID NO: 6 in an *in-vitro* system, and
- (b) obtaining the polypeptide from the cell, the culture medium or the *in-vitro* system.

33. A method of finding a chemical compound which binds to a polypeptide according to Claim 15 or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps:

- (a) contacting a host cell comprising a nucleic acid which encodes a polypeptide with the bioactivity of a zeta-carotene desaturase, comprising the amino acid sequence of SEQ ID NO: 6, a polypeptide according to Claim 15 or a polypeptide with the bioactivity of a phytoene desaturase with a chemical compound or a mixture of chemical compounds under conditions which permit the interaction of a chemical compound with the polypeptide, and
- (b) determining the chemical compound which binds specifically to the polypeptide.

34. A method of finding a compound which modifies the expression of polypeptides according to Claim 15 or a polypeptide with the bioactivity of a phytoene desaturase, comprising the following steps:

- (a) contacting a host cell containing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: with a chemical compound or a mixture of chemical compounds,
- (b) determining the polypeptide concentration, and

- (c) determining the compound which specifically influences the expression of the polypeptide.

35. An organism selected from transgenic plants, parts of plants, protoplasts, plant tissues and plant propagation materials, wherein the organism comprises an intracellular concentration of a polypeptide according to Claim 17 which is increased or reduced in comparison with the corresponding wild-type cells after introducing a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

36. An organism selected from plants, parts of plants, protoplasts, plant tissues or plant propagation materials, wherein the organism comprises a polypeptide according to Claim 15 whose bioactivity or expression pattern is modified in comparison with the corresponding endogenous polypeptides.

37. A method of generating plants, parts of plants, protoplasts, plant tissues or plant propagation materials according to Claim 25, comprising the step of modifying by endogenous mutagenesis a regulatory region which naturally controls, in plant cells, the transcription of a nucleic acid which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 6.

38. A nucleic acid comprising a sequence selected from the group consisting of:

- (a) the sequences of SEQ ID NOS: 1, 3 and 5,
- (b) sequences encoding polypeptides which comprises the amino acid sequences of SEQ ID NOS: 2, 4, and 6, and
- (c) sequences which, owing to the degeneracy of the genetic code, encode the same amino acid sequence as the amino acid sequences of SEQ ID NOS: 2, 4, and 6.